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RESEARCH ARTICLE

Influence of phenolic compounds and flavonoids on the colour and antioxidant activity of *Melipona beecheii* honey from deciduous forest of Yucatan, Mexico

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ABSTRACT

The *Melipona beecheii* honey has bioactive compounds with high antioxidant activity that influence its colour. This research evaluated the influence of phenolic compounds and flavonoids on the colour and antioxidant activity of *M. beecheii* honeys. Forty-three honey samples from different site located in the low deciduous forest of Yucatan, Mexico, were extracted during the harvest and post-harvest seasons (2020 – 2021). The Pfund method was used to measure colour, Folin-Ciocalteu method for phenols measurement, aluminum chloride method for flavonoids determination, and ABTS and DPPH IC_{50} assay to determine antioxidants. The predominant colour of the honey was extra light amber and light amber at both seasons. Phenols ranged from 780 to 1317 mg/kg GAE; flavonoids from 36 to 55 mg/kg CE and antioxidant were 675 to 1161 μmol/kg TEAC by the ABTS and 361.8 to 173.0 mg/mL by the DPPH IC_{50}. Phenols and flavonoids were correlated with colour and radical scavenging activity, indicating the influence of these compounds with the darker colour and higher antioxidant activity of honey. The statistically significant difference ($P<0.05$) between seasons could be associated with the botanical origin with great diversity of melliferous flora present in the low deciduous forest of the Yucatan Peninsula.

Keywords: Antioxidant; Colour; Flavonoids; Honey; Melipona beecheii

INTRODUCTION

Honey is a natural substance produced by bees, whether of the genus *Apis* or *Meliponini*, from the nectar of flowers or from secretions of living parts of plants that the bees collect, transform, deposit, dehydrate and store in wax combs in the case of *Apis*, and in pots or cerumen "botijas" in the case of *Meliponini*.

Honey is essentially a saturated solution of different sugars, the main ones being glucose and fructose which together account for 85 to 96% of the total. Residual carbohydrates are disaccharides, trisaccharides and oligosaccharides (Costa et al., 2015).

Other substances found in honey include acids, proteins, mineral, and trace components. The wide variety of minor components includes phenolic acids and flavonoids, ascorbic acid, enzymes, carotenoids, and Maillard reaction products, all of which are contributors to the antioxidant potential of honey (Abu Bakar et al., 2017). However, flavonoids and phenolic acids are considered to represent most phenolic compounds or plant polyphenols and, therefore, the main antioxidants in honey. Furthermore, it has been reported that the polyphenols have also to affect some of honey such as appearance of honey, flavor and particularly its colour (Alvarez et al., 2010)

Honey produced by stingless bees has a high content of total phenolics and, consequently, a higher antioxidant capacity compared to other types of honeys (Al-Hatamleh et al., 2020).

The chemical composition and antioxidant properties of honey are directly related to its geo-botanical origin and environmental conditions after honey extraction,

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processing, and storage, which have produced interest in identifying and quantifying these bioactive compounds, not only to determine honey quality, but also to use them as markers of geographic origin (Biluca et al., 2016).

Two species of *Melipona*, *M. beecheii* and *Melipona yucatanica,* inhabit the Yucatan Peninsula, Mexico; however, the latter is uncommon and there are few records of its use in meliponiculture (González, 2012) compared to the breeding of *M. beecheii,* whose honey was widely exploited by the Mayas within traditional medicine and which in the last 15 years has had a resurgence (Bratman, 2020) due to the growing demand for natural products and with functional properties.

The Yucatan Peninsula is characterized by vegetation rich in melliferous and pollen flora that is important for beekeeping. According to what is published by the National Forestry Commission in the state of Yucatan, only four ecoregions are distinguished in the state of Yucatan: low deciduous forest (northwestern plain), medium subdeciduous forest (central Yucatecan plain), medium and high subperennifolia forests, in addition to wetlands of northern Yucatan and the Caribbean. Each of these regions has a floristic diversity that supports beekeeping and meliponiculture, obtaining honeys with diverse physicochemical characteristics and distinctive organoleptic characteristics (IEFYS, 2014)

The low deciduous forest is important not only for its particular floristic richness, with more than 50 % of the genera and species identified in lowland forest, but also for its high proportion of endemic species (IEFYS, 2014). Observing a diversity of native plant species, approximately 2 400 species recorded to date, of which 600 are melliferous and 30 of them considered of great beekeeping importance (Castillo et al., 2016). And it is there where the apibotanical cycle, which includes the seasons of pre-harvest from October to December, harvest from January to May and post-harvest from June to September (Alfaro et al., 2010), has a close relationship with environmental conditions, which affect the flowering of plants and honey production, thus presenting wide fluctuations throughout the year (Cruz, 2017).

However, most research has focused on honey produced by the European honeybee *A. mellifera* and, until recently, attention has been paid to honey from stingless bees. Therefore, the objective of this study was to determine the influence of total phenolics and flavonoids on the colour and antioxidant activity of honey produced by *M. beecheii* extracted during the harvest and post-harvest seasons from meliponaries located in the low deciduous forest of the state of Yucatan.

MATERIALS AND METHODS

Sample collection

From February 2020 to May 2021, 43 samples of *M. beecheii* honey were collected from meliponaries located in 18 municipalities located in the low deciduous forest region.

Nineteen samples were obtained in the post-harvest season (June, July, August, September, and October 2020) and 24 in the harvest season (February, March, April, May and December/2020 and January, February, March, April and May/2021) according to the apibotanical calendar of the Yucatan Peninsula.

Honey extraction was carried out by each honeybee farmer of native bees by pouring in the case of "jobon", or with a syringe or pipette in the case of boxes.

Samples were collected, transported in 500 mL polyethylene containers, isolated from light in an isothermal container and kept refrigerated at 4 °C until analysis in the INIFAP laboratories. The samples obtained were subjected to the following determinations:

Colour

The colour intensity of the honey samples was measured according to the Pfund classifier scale. Honey samples were homogenized and air bubbles were removed. Approximately 2 mL of honey was transferred into a cuvette with a 10 mm light passage. The cuvette was introduced into a HI 96785 colour photometer (HI 96785, Hanna Instruments, Cluj County, Romania) previously calibrated with an analytical grade glycerol standard reference. Colour grades were expressed in millimeters (mm) (scale 0-144 mm) Pfund grades when compared to an analytical-grade glycerol standard and colour on the scale of water white to dark amber (NOM, 2018). Measurements were performed in triplicate for each sample.

Determination of total phenolics content

The phenolic compounds were determined using the Folin-Ciocalteu method, based on the reduction of phosphor-wolframate-phosphomolybdate complex by phenolics to a blue reaction product according to the modified method reported by Al et al. (2009). 500 µL of a honey solution (1g honey in 10 mL of distilled water) was taken in a beaker and mixed with 2.5 mL of Folin-Ciocalteu reagent (Sigma Aldrich) (0.2 mol/L) for 5 min on a magnetic plate at room temperature. Subsequently, 2 mL of a Na_2CO_3 solution (7.5%) was added and incubated at room temperature in dark conditions for 2 h. The dispersion was stirred on a magnetic plate at room temperature for 3 h. After the extraction the dispersion was centrifuged at 1200 *x g* for 20 min at 10 °C. The supernatant was filtered, refrigerated

at 4 °C and protected from light until analysis. After, the sample was placed in a plastic cell the absorbance was read at 760 nm in VE-5100UV spectrophotometer (Velab, Mexico) using distilled water as blank. A standard solution of gallic acid (1 g/L) (Sigma Aldrich) was used to perform a calibration curve (1 – 100 µg/mL; R^2 = 0.9995). The total amount of phenolic compounds was determined in milligrams of gallic acid equivalents (GAE) per kilogram of honey.

Determination of total flavonoids content

The total flavonoid content in each honey sample was measured using the technique proposed by Moniruzzaman et al. (2013), based on a colourimetric assay. One g of honey was weighed and dissolved in 1 mL of distilled water. At the baseline, 0.3 mL of NaNO2 (5%, w/v) was added. After five min, 0.3 mL of AlCl3 (10% w/v) was added, followed by the addition of 2 mL of NaOH (1 M) 6 min later. The volume was then increased to 10 mL by the addition of 2.4 mL distilled water. For sample, a 1 mL aliquot of the honey solution was taken, mixed with 4 mL of distilled water and 0.3 mL of $NaNO_2$ (5% w/v) was added. After allowing to stand for 5 min, 0.3 mL of $AlCl_3$ (10% w/v) was added followed by the addition of 2 mL of NaOH (1 mol/L) 6 min later. The volume was then brought to 10 mL by adding 2.4 mL of distilled water. The mixture was stirred in a vortex to subsequently read the absorbance at a wavelength of 510 nm in VE-5100UV spectrophotometry equipment (Velab, Mexico). Distilled water was used as blank. A standard solution of catechin (Sigma Aldrich) (1 g/l) was used to perform a calibration curve (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL; R^2 = 0.999), expressing the results in catechin equivalents (CEq) in miligrams per kilogram of honey.

Antioxidant activity

2, 2'-azino-bis-(3-ethylbenzothiazolin-6-sulfonic acid (ABTS)

Antioxidant activity was determined by a modification of the spectrophotometric technique proposed by Contreras et al. (2020). The $ABTS^{\bullet+}$ radical cation was produced by reacting 2, 20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) with potassium persulfate. To prepare the stock solution, ABTS was dissolved at a 2 mM concentration in 50 mL phosphate-buffered saline (PBS) prepared from 4.0908 g NaCl, 0.1347 g KH_2PO_4, 0.7098 g Na_2HPO_4, and 0.0749 g KCl dissolved in 500 mL ultrapure water. If pH was lower than 7.4, it was adjusted with NaOH. A 70 mM $K_2S_4O_8$ solution in ultrapure water was prepared. The ABTS radical cation was produced by reacting 10 mL ABTS stock solution with 40 µL $K_2S_2O_8$ solution and allowing the mixture to stand in darkness at room temperature for 16 h before use. The radical was stable in this form for more than 2 days when stored in darkness at room temperature. Antioxidant compound content in the sample was analyzed by diluting the $ABTS^{\bullet+}$ solution with PBS to an absorbance of 0.8 ± 0.03 measured at a wavelength of 734 nm nm in VE-5100UV spectrophotometer (Velab, Mexico). After adding 990 µL diluted $ABTS^{\bullet+}$ solution to 10 µL of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid standard in PBS, absorbance was read at room temperature exactly 6 min after initial mixing g PBS instead of the honey solution as blank. All determinations were carried out in triplicate. The percentage decrease in absorbance at 734 nm was calculated and plotted as a function of the antioxidant concentration of Trolox for the standard reference data. A calibration curve was performed with Trolox solution (0.5 - 3.5 mol/L; R^2 = 0.9998). The To calculate the Trolox equivalent antioxidant coefficient (TEAC), the slope of the absorbance inhibition percentage vs. antioxidant concentration plot was divided by the slope of the Trolox plot. The results were expressed as antioxidant activity equivalent micromol units of Trolox (TEAC) per kilogram of honey.

2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH assay was carried out using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical according to Al-Farsi et al. (2018) with some modifications. Honey samples were diluted in distilled water at concentration of 50 to 250 mg/mL, and from each dilution 0.3 ml was mixed with 2.7 mL of DPPH (Sigma Aldrich) (0.02 g/L in 95% methanol). The mixtures were vortexed, left in the dark at room temperature for 60 min and absorbance was measured at a wavelength of 517 nm in spectrophotometer VE-5100UV (Velab, Mexico). Measurement was performed at least in triplicate.

The radical inhibition activity (RSA) was calculated as a percentage of DPPH using the following equation: % RSA = (Abs 0 - Abs 1/Abs 0) x 100.

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of the honey sample. The IC_{50} (concentration providing 50% inhibition, 50% RSCA) values were calculated using the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging effect. Samples were tested in a range of concentrations from 50 to 250 mg/mL.

Statistical analysis

Samples were analyzed at least in duplicate for the same period to ensure uniform conditions, presenting for the study variables the descriptive statistics mean and standard deviation.

After verifying that the results obtained did not meet the postulates of normality, the post-harvest and harvest

groups were compared using the Mann-Whitney U statistic and the Spearman test was used to measure the correlation coefficient to find the association between the variables of colour, phenolics, flavonoids and antioxidant activity.

The R statistical package (v. 4.0.2) was used for data analysis (https://rstudio.com/).

RESULTS

Colour

The predominant colours of the analyzed honey samples were extra light amber (50.0 % and 31.6 %) and light amber (25.0 % and 47.4 %) for the harvest and post-harvest seasons, respectively. Statistically significant differences ($P < 0.0.5$) were observed between the production seasons, being the honeys from the harvest (42.1 mm Pfund) lighter than the honeys from the post-harvest (71.2 mm Pfund) (Table 1).

Phenolics compounds and flavonoids contents

The total phenolic content in this study presented an average 780 mg/kg during the harvest season and 1317 mg/kg GAE during the post-harvest season. Meanwhile, the flavonoid content was 36.0 mg/kg CE for harvest season honeys and 55.0 mg/kg CE for post-harvest season (Table 2).

Antioxidant activity

The antioxidant activity by the ABTS method of honey showed a significant statistical difference ($P < 0.05$) between harvest (675 µmol/kg TEAC) and post-harvest (1161 µmol/kg TEAC). With respect to DPPH free radical inhibition activity by means of the IC_{50} parameter, a lower value in honey indicates a higher antioxidant capacity, that is, to neutralize free radicals. The average values in this study were 361.8 mg/mL for harvest and 173.0 mg/mL for post-harvest season (Table 3).

Correlation between colour, phenolics compounds, flavonoids and antioxidant activity

The correlation established between colour, phenolics, flavonoids and antioxidant activity at and harvest and post-harvest seasons is presented in Table 4, to explore the possible relationships between these values.

The first general observation is that there was a positive correlation between colour, phenolics and flavonoids, as

Table 1: Colour in honey samples of *M. beecheii* at harvest and post-harvest seasons

Harvest			Post-harvest		
Sample ID	Pfund [mm]	Colour	Sample ID	Pfund [mm]	Colour
01	38.5	Extra light amber	07	44.0	Extra light amber
02	50.0	Extra light amber	08	77.0	Light amber
03	50.0	Extra light amber	09	150.0	Dark amber
04	38.0	Extra light amber	10	51.5	Light amber
05	67.5	Light amber	11	66.0	Light amber
06	37.0	Extra light amber	12	45.5	Extra light amber
25	53.5	Light amber	13	67.0	Light amber
27	84.5	Amber	14	50.0	Extra light amber
28	5.0	Water white	15	54.5	Light amber
29	17.0	Extra white	16	39.0	Extra light amber
30	42.0	Extra light amber	17	89.5	Amber
31	37.0	Extra light amber	18	65.5	Light amber
32	42.5	Extra light amber	19	63.0	Light amber
33	35.0	Extra light amber	20	46.0	Extra light amber
34	62.0	Light amber	21	80.5	Light amber
35	14.5	Extra white	22	150.0	Dark amber
36	18.5	White	23	81.0	Light amber
37	40.0	Extra light amber	24	85.5	Amber
38	19.0	White	26	47.5	Extra light amber
39	75.5	Light amber			
40	45.0	Extra light amber			
41	70.5	Light amber			
42	32.0	White			
43	37.0	Extra light amber			
Mean±SD	42.1±19.9[a]	Extra light amber	Mean±SD	71.2±31.7[b]	Light amber
Min.	5.0		Min.	39.0	
Max.	84.5		Max.	150.0	

[a-b]Different letters in the same row indicate significant statistical difference ($P<0.05$)

Table 2: Phenolics and flavonoids in honey samples from *M. beecheii* at harvest and post-harvest seasons

Harvest			Post-harvest		
Sample ID	Phenolics [mg/kg]	Flavonoids [mg/kg]	Sample ID	Phenolics [mg/kg]	Flavonoids [mg/kg]
01	867	22	07	1034	38
02	1114	34	08	1248	62
03	963	44	09	1594	52
04	739	33	10	1253	46
05	1563	45	11	1474	52
06	1005	42	12	969	38
25	599	38	13	1149	50
27	980	51	14	1025	36
28	300	13	15	1149	45
29	356	25	16	951	33
30	596	30	17	1253	62
31	562	30	18	1613	45
32	577	34	19	1179	43
33	663	45	20	990	37
34	1260	51	21	1677	66
35	354	29	22	2479	144
36	326	30	23	1502	77
37	946	43	24	1652	80
38	384	12	26	826	41
39	1080	70			
40	866	36			
41	1253	49			
42	645	27			
43	691	26			
Mean±SD	780±337[a]	36±13[a]	Mean±SD	1317±385[b]	55±26[b]
Min.	300	12	Min.	826	33
Max.	1563	70	Max.	2479	144

[a-b]Different letters in the same row indicate significant statistical difference (P<0.05)

well as the antioxidant activity by the ABTS technique, while the relationship with the DPPH technique due to the calculation of the IC_{50}, whose low value indicates a greater antioxidant effect with a lower concentration of compounds.

The strongest correlation in honeys extracted at harvest season was between colour, phenolics and ABTS, suggesting that antioxidant activity is increased in darker honeys, and both associated with the concentration of phenolic compounds. Instead, in post-harvest honeys, the highest correlation is observed between colour and flavonoids, indicating that darker honeys can be attributed to the presence of a high amount of these compounds.

DISCUSSION

Colour

The results of this research agree with that reported by Moo et al. (2015), who reported 37% of honeys classified as light amber, 26 % extra light amber, the rest were white (14.8 %), amber (18.5 %) and dark amber (3.7 %) in *M. beecheii* honey from the Yucatan Peninsula, Mexico. Also, for *M. beecheii* honey, Damaceno do Vale et al. (2018), reported 69 % light amber and 31% extra light amber, in honeys from Brazil; while May et al. (2022) refer as average extra light amber colour (37.05±22.1 mm Pfund) for honeys from Mexico and Guatemala. However, our results differ from those described by Gutiérrez et al. (2009) and Grajales et al. (2018), who refer white colour (25.50 ± 24.56 mm Pfund) for honeys from Guatemala and dark amber colour (136.0 mm Pfund) for honeys from Chiapas, Mexico, respectively, being that in this study only 3 samples were white coloured at harvest season and 2 dark amber coloured post-harvest.

The statistically significant difference observed between the production seasons is consistent with Alfaro et al. (2010), who reported for *A. mellifera* honeys from Yucatan, white to light amber (22-54 mm Pfund) for honeys from the harvest (December-February) and extra light amber to light amber (38-68 mm Pfund) for honeys from the post-harvest (March-May).

The differences in colour of the honeys may be due to the different nectar-polliniferous species that bloom throughout the year in the low deciduous forest of the Yucatan Peninsula. It has been reported that both the

Table 3: Antioxidant activity in *M. beecheii* honey samples at harvest and post-harvest seasons

Harvest			Post-harvest		
Sample ID	ABTS [µmol TEAC/kg]	DPPH [IC_{50} mg/mL]	Sample ID	ABTS [µmol TEAC/kg]	DPPH [IC_{50} mg/mL]
01	734	242.2	07	914	243.6
02	907	255.2	08	1250	182.3
03	879	200.2	09	845	43.7
04	650	221.0	10	1012	190.4
05	1119	175.9	11	985	168.9
06	506	231.6	12	764	193.0
25	534	347.3	13	1217	193.1
27	888	192.7	14	624	253.5
28	108	1224.0	15	1019	222.4
29	188	1056.5	16	1343	181.6
30	616	242.2	17	970	198.6
31	568	319.5	18	1043	192.0
32	605	561.6	19	1085	201.7
33	592	545.1	20	963	225.6
34	1268	99.1	21	1442	140.9
35	241	516.7	22	2559	50.0
36	309	545.1	23	1540	119.9
37	1038	213.8	24	1697	113.2
38	355	562.6	26	789	172.6
39	1017	174.6			
40	951	224.3			
41	1079	192.8			
42	507	171.0			
43	553	168.2			
Mean±SD	675±315[a]	361.8±280.7[a]	Mean±SD	1161±436[b]	173.0±57.3[b]
Min.	108	99.1	Min.	624	43.7
Max.	1268	1224.0	Max.	2559	253.5

[a-b]Different letters in the same row indicate significant statistical difference (P<0.05)

Table 4. Correlation between colour, phenolics compounds, flavonoids, and antioxidant activity in *M. beecheii* honey samples

Harvest					
	Phenolics	Flavonoids	Colour	ABTS	DPPH
Phenolics	1.00	0.75	0.80	0.86	-0.75
Flavonoids	0.75	1.00	0.79	0.74	-0.54
Colour	0.80	0.79	1.00	0.86	-0.61
ABTS	0.86	0.74	0.86	1.00	1.00
DPPH	-0.75	-0.54	-0.61	-0.67	1.00
Post-harvest					
	Phenolics	Flavonoids	Colour	ABTS	DPPH
Phenolics	1.00	0.86	0.85	0.59	-0.61
Flavonoids	0.86	1.00	0.91	0.64	-0.68
Colour	0.85	0.91	1.00	0.48	-0.61
ABTS	0.59	0.64	0.48	1.00	-0.51
DPPH	-0.61	-0.68	-0.61	-0.51	1.00

Fabaceae and Convulvulaceae families present a high concentration of tannins and alkaloids which is associated with a darker colour of the honeys (Otmani et al., 2019). According to the phenology of plant species in the months of June-September, fabaceae such as *Acacia gaumeri* (box katsim), *Caesalpinia gaumeri* (kitam che'), *Lysiloma latsiliquum* (tsalam), *Havardia albicans* (chukum), *Apoplanesia paniculada* (chulúul) bloom; and from October to December, flowering convolvulaceae such as *Distimake aegyptia* (tso'ots kàab) and *Jacquemontia pentantha* (ak'il xíiw) and herbaceous plants of the genus *Senna* spp. (Alfaro et al., 2010), which explains the darker colours found in the post-harvest honeys.

Almeida et al. (2013), found a statistically significant difference between honeys of *A. mellifera* (26.67 ± 0.58 mm Pfund) and honeys of *M. subnitida* (7.00 ± 0. 01 mm Pfund) from northeastern Brazil, referring that the palynological profile of the two types of honeys in that study was completely different, suggesting that the two bee species do not visit the same plants; however, contrary to those results, Alvarez et al. (2018) reported no difference between honeys of *A. mellifera* (37.35 ± 6.52) and *M. beecheii* (41.65 ± 7.68) from Cuba. The colours of the honeys obtained in this study were darker than reported by Almeida et al. (2013), in both species but similar to those reported by Alvarez et al. (2018), indicating that the colour is associated with the flowering from which the bees obtained the nectar.

The colour of honey is an important sensory attribute with respect to consumer preference. In general, consumers around the world prefer dark, yellow, and amber *A. mellifera*

honeys (Altmann, Trinks and Mörlein, 2023); however, the results obtained in this study indicate that honey produced by *M. beecheii* tends to present light colours without this meaning that the dark colour in this type of honey indicates that it is of inferior quality.

Phenolics compounds and flavonoids contents

The mean value of total phenolic content in this research was similar to that reported for *M. beecheii* honeys by Silva et al. (2013), 1103.3 ± 0.11 mg/kg GAE in Brazil and higher to that reported by Sánchez et al. (2019) with 485.3 ± 39.2 mg/kg GAE in Tabasco, Mexico and Ruiz et al. (2017) with 632.2 mg/kg GAE in Yucatan, Mexico, but lower than determined by Nweze et al. (2017) 3719.8 ± 141.8 mg/kg GAE for *Melipona* sp. in Nigeria.

In investigations where *Apis* and *Melipona* honey samples were collected in the same geographical area Alvarez et al. (2018), found a statistical difference ($P < 0.05$) between the phenolics of *A. mellifera* and *M. beecheii* honey with 543.0 ± 71.9 and 943.9 ± 145.5 mg/kg GAE, respectively. Contrary case to that reported by Nweze et al. (2017) who in their research determined 4391.6 ± 290.6 mg/kg GAE for *A. mellifera* honey and 3719.8 ± 141.8 mg/kg GAE for *Melipona* sp, as well as Ramón et al. (2020) with 946.0 ± 15.0 mg/kg and 515.0 ± 20.0 mg/kg GAE for *A. mellifera* and *M. beecheii* honey, respectively.

This variability in the concentration of phenolics was also observed in the honeys analyzed in this study, which could be due to a difference in the phenolic profiles of the honeys, attributed to divergences in the origin of the nectar and the bioactive components transferred from the plants (Ramón et al., 2020), that is, although the honeys were collected in the same area, they may present differences depending on the vegetation that is flowering in the area. It is worth mentioning that this influence of the floral source on the phenolics and flavonoids in honeys from the same geographical region had already been reported by researchers such as Silva et al. (2013).

The total flavonoid content in the present study is consistent with that reported by Alvarez et al. (2018) for *M. beecheii* honey with 41.9 ± 3.7 mg/kg CE, but lower than that detected by Nweze et al. (2017) 86.3 ± 4.6 mg/kg CE for *Melipona* sp honey and higher 29.7 ± 9.0 mg/kg QE than that reported by Sousa et al. (2016) for stingless bee honeys.

Alvarez et al. (2018), Nweze et al. (2017) and Sousa et al. (2016), determined that flavonoids in their investigations were higher in bee honeys from the *Meliponini* tribe relative to *A. mellifera*, which significantly contributes to the recognized therapeutic benefits in *M. beecheii* honey due to the relationship with its antioxidant activity.

The content of phenolic compounds, unavoidable components in the vegetation present in the low deciduous forest, increases during the summer, which is attributed to the fact that most of these compounds fulfill defense functions against predators, pathogens, and solar irradiation (Salminen et al., 2004) characteristic of this season.

Because the biosynthesis of phenolic compounds, flavonoids and non-flavonoids, is regulated by different enzymes, depending on the plant species, its needs and the oxidative stress to which they are subjected (Dezmirean et al., 2017), the environmental and climatic conditions where the plants grow directly impact the composition of the nectar that the bee forages for honey production (Rodríguez, Escuredo and Seijo, 2015) so in addition to the time of year, another cause for the higher content of polyphenols and flavonoids found in honeys collected during the post-harvest season, is that according to the National Water Comission in Mexico (CONAGUA) report in 2020 Yucatán was one of the entities that observed its warmest year, with maximum temperatures of up to 42 °C, compared to 40 °C and 39 °C in 2019 and 2018 respectively (CONAGUA, 2021), which would cause an increase in phenolic compounds in plants subjected to water stress (Bautista et al., 2016).

Antioxidant activity

Phenolics are known as a class of bioactive compounds that show strong antioxidant effects *in vitro* and *in vivo*. In this study, two antioxidant assays, $ABTS^{\bullet+}$ and DPPH IC_{50}, were used to determine the antioxidant capacity of honey. The principle of both assays is similar, the interference of non-physiological free radicals (Oliveira et al., 2017), which allows them to be used in a wide pH range and in multiple media to determine antioxidant capacities (Majid, Ellulu and Abu Bakar, 2020).

The antioxidant activity by the ABTS method of honey is in accordance with what is described by Gutiérrez et al. (2009) with 873.8 ± 129.2 µmol/kg TEAC for *M. beecheii* honey in Guatemala, but higher than that reported for other meliponinos species in Brazil by Sousa et al. (2016) with 232 - 816 µmol/kg TEAC.

Several studies have compared the antioxidant properties of honey produced by stingless bees with that produced by *A. mellifera*. However, when comparing with what has been published for *A. mellifera* honey produced in Mexico, it can be observed that the results obtained in the present investigation are lower than those reported by Rodríguez et al. (2012) and Contreras et al. (2020), with values up to 2927.4 ± 44.6 and 183.09 ± 22.91 µmol/kg for monofloral honeys, but were higher to what is mentioned by Tapia et al. (2017) with 121.9 – 527.9 µmol/kg TEAC and Contreras

et al. (2020) with 165.6 µmol/kg TEAC for multifloral honeys. The above provides support that botanical inference is more relevant to entomological inference in honey antioxidant activity.

With respect to DPPH free radical inhibition activity by means of the IC_{50} parameter, the values obtained in this research presented a lower inhibitory effect when compared to that referred by Silva et al. (2013) with values from 10.60 to 12.90 mg/mL for *M. subtinida* honey from Brazil; Oliveira et al. (2017) with 25.39 mg/mL and 51.44 mg/mL for *M. q. quadrifasciata* and *M. scutellaris*, respectively and by Aroucha et al. (2019) 141.86 mg/mL for *M. subnitida*.

The IC_{50} in this study shows similar results to that reported by Nascimento et al. (2018) and Shamsudin et al. (2019) for *A. mellifera* honeys with results up to 294.26 mg/mL and 202.15 mg/mL. However, Shamsudin et al. (2019) when comparing honey from stingless bees and *A. mellifera*, through this technique, confirmed that honey from stingless bees presented a lower IC_{50} value, that is, a higher antioxidant activity in the compounds present, with respect to honey from *A. mellifera*.

Even though it has been shown that the antioxidant activity of honey is associated with the content and type of phenolic compounds derived from the variety of resources used by bees in each season of the year, the antioxidant mechanism of honey also includes peptides, organic acids, enzymes products of the Maillard reaction, and possibly other minor components, in which interactions among them can affect the total antioxidant activity, producing synergistic or antagonistic effects (Herken et al., 2010) which was confirmed by Shamsudin et al. (2019), describing a marked difference in chemometric analysis and amino acid profile between honeys from *A. mellifera* and stingless bee species *Heterotrigona itama*.

Correlation between colour, phenolics compounds, flavonoids and antioxidant activity

The correlation obtained in this study was similar to the one described by Al-Farsi et al. (2018) and Khongkwanmueang et al. (2020) for *A. mellifera* and *Tetragonula laeviceps* honeys, respectively. Not so with what is reported by Shamsudin et al. (2019) for *Heterotrigona* honey who found no significant correlations between honey colour and phenolics or flavonoids values, suggesting that these results colour is not influenced by these compounds in Malaysian stingless bee honey (Herken et al., 2010). However, most research has shown a linear relationship between the concentration of phenolics with honey colour and antioxidant activity (Herken et al., 2010) associated with botanical origin rather than geographic location (Cheung et al., 2019).

CONCLUSIONS

This study correlate phenolics compounds and flavonoids with the colour and antioxidant activity of *M. beecheii* honey produced in the deciduous forest of Yucatan, Mexico at different seasons of the year. Significant differences between the harvest and post-harvest seasons of the honeys analyzed suggest different botanical origins with a great diversity of nectariferous flora despite coming from the same ecosystem, as well as the environmental conditions to which they were subjected before harvesting. Positive associations were found between phenolics and flavonoids with colour and antioxidant activity by the ABTS technique, and negative for the DPPH IC_{50} technique, which suggests that darker honeys have higher polyphenol content and therefore a higher antioxidant activity.

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